

Final Report for AOARD Grant AOARD104131

“Sensory coordination of insect flight”

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Period of Performance: 1/August/2010 – 1/August/2011

Summary

In the past year, we have been working on the following main projects with reference to our work plan:

Antennal positioning in moths and freely flying bees:

1. Latency studies: We had initially planned to conduct Transmission Electron Microscopy (TEM) imaging to directly visualize the synapses that connect the sensory neurons to the motor neuron. This might take a little longer as the training time on TEM is fairly extended. However, we approached this question from another angle: is there information flow through the sensorimotor circuits and if so, can the latencies of this circuit inform us about the connectivity. We have conducted detailed measurements of these latencies. We are currently in the process of writing this manuscript.

2. An extended survey of the Bohm's bristles: Our preliminary data suggested that the Bohm's bristle system is conserved across many insects and sought to demonstrate that this system may be conserved across diverse insect taxa. We have now surveyed representatives from several insect clades using Scanning Electron Microscopy

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14. ABSTRACT This is the final report of a project that studied antennal positioning in moths and freely flying bees, location of odor sources in the fruit fly, coordination of wings and halteres in the soldier fly, and landing behavior in the housefly.					
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(SEM), and do indeed find that most (but not all) of them have Bohm's bristles, or mechanosensory structures that resemble these bristles.

Location of odor sources in the fruit fly, *Drosophila melanogaster*

We were able to complete a full set of experiments in which flies were challenged to find odor sources in a wind-tunnel, in presence of a constant airflow but variable separation of visual and olfactory cues. These experiments suggest that flies pay close attention to the visual cues during the process of finding odor sources, and indeed, their flight towards an odor sources is often biased by these cues.

Coordination of wings and halteres in the soldier fly, *Hermetia illucens*

We have conducted a detailed set of experiments on trying to uncover both the wing-wing and wing-haltere connectivity in soldier flies. The first phase of this project has been completed, and we are now in the process of writing a manuscript on this study.

Landing behavior in the housefly, *Musca domestica*

We were previously able to design precise assays to elicit landing in the housefly, *Musca domestica* such that they could be filmed with two cameras equipped with macro-lenses. These films have now been quantified.

Introduction

Our recent work has been focused on the question of how insect nervous systems combine information from multiple sensory modalities to generate fast responses, as required during flight. To this end, we have taken a comparative approach and have developed several systems of study including moths, honeybees, soldier flies and houseflies. Each system is associated with a specific project and research question, but together they fall within the gamut of the above mentioned goal. Moreover, we are also conducting investigations at diverse levels of organization from individual neurons to whole animal behavior, and trying to understand how the mechanisms of these behaviors can be explained from a neuromechanical perspective. First, we find that fast flight-related mechanosensory feedback from antenna shows direct neural

connectivity with the antennal motor neurons. We have now established this over the past three years using behavioral, neuroanatomical and neurophysiological evidence. In the process, we also discovered that this elegant mechanosensory-motor feedback loop also integrates visual information and are now undertaking to more closely study this aspect. Second, we show that in cases where closely coordination of various appendages is necessary at very rapid rates (\sim a few hundred Hz), the system relies on direct mechanical linkages for speed. Third, we show that at the whole animal level such precise coordination may be very critical for fine-tuned but fast aerial maneuvers. Through these efforts, we hope to put together a comprehensive picture of how flying insects use multiple sensory inputs to actuate their appendages during fast maneuvers.

Experiments, Results and Discussion

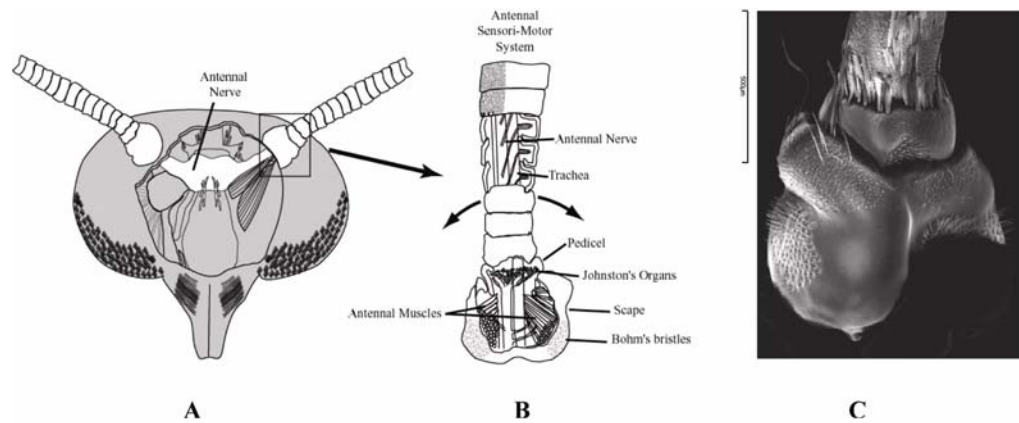


Fig 1: Antennal Anatomy A. Frontal view of antenna and brain connected by antennal nerve. B. Morphology of the antenna. C. Scanning electron micrograph of antennal base. Black arrows point to Bohm's bristles.

Antennal positioning response in the hawk moth, *Daphnis nerii*:

Over the past few years, we have used high-speed videography to characterize the antennal positioning behavior in the hawk moth, *Daphnis nerii*. We also identified Bohm's bristles (Fig 1 A-C) located on the surface of basal antennal segments as the candidate mechasensors that sense the changes in antennal positions and communicate this information to an area of the brain called the Antennal

Mechanosensory and Motor Centre (AMMC). Using fluorescent dye fills of the nervous system and confocal microscopy, we showed that in the AMMC, the sensory arbor of the Bohm's bristle neurons arborize on the dendritic arbors of the antennal motor neurons. This suggested the hypothesis that the connections were monosynaptic and that the antennal positioning response mediated through the Bohm's bristles was a classic reflex arc.

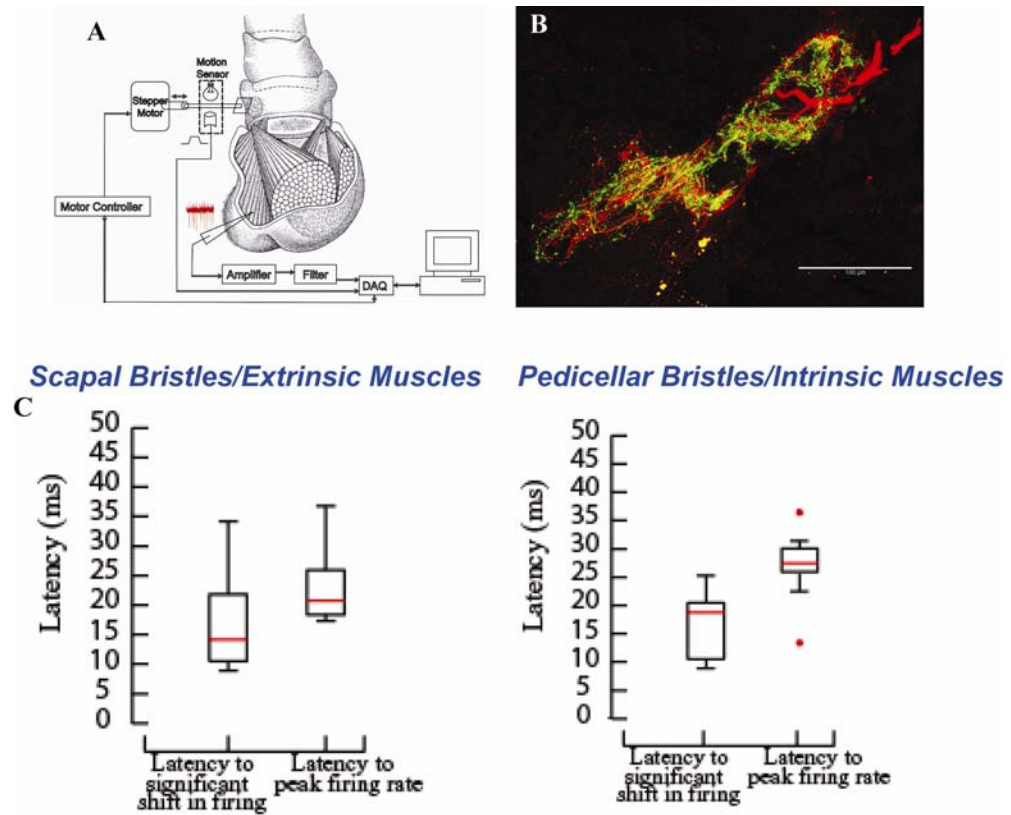


Figure 2: (A) Electrophysiological setup to measure the response latencies of antennal muscles to stimulation of Bohm's bristles. (B) Overlap of arbors between axonal projections of mechanosensory Bohm's bristles (green, Fluorescein dextran) and antennal motor neurons (Red, Rhodamine dextran). (C) Response latencies of extrinsic antennal muscles following scapal bristle stimulation (left panel) and latencies of intrinsic antennal muscles following pedicellar bristle stimulation (right panel). Significant shift in patterning (left box plot in each panel) signifies change of activity by 5 standard deviations from baseline whereas right panel signifies latency to peak response.

Over the last year, we were able to test this hypothesis using electrophysiological

means. Briefly, we set up an electrophysiological rig that allowed us to specifically stimulate the Bohm's bristles on the insect antennae while simultaneously conducting electromyogram (EMG) recordings of their antennal muscle activity. The time duration between the instant of stimulus and the peak response (or other measures of significant response over baseline) was defined as the latency of the antennal positioning response. By our estimation, this latency is approximately 15 ms, which suggests a direct connection between the sensory and motor neurons, accounting also for the neuromuscular junction.

During the course of these experiments, we also discovered that antennal muscles responded to visual stimuli, leading to the intriguing possibility that this monosynaptic mechanosensory-motor loop also integrates information from the visual system. We have recently begun pursuing these experiments and hope to decipher the underlying mechanisms of visual-mechanosensory-antennomotor response integration system, as an illustrative example of how nervous systems integrate multi-sensory information. We feel that this system is especially handy as a model system because all the components – the sensory and motor structures as well as the internal connectivity is easily accessible for experiments. This work, which was initially seeded by the AOARD small grants, has recently received funding from Air Force Office of Scientific Research for further investigation.

Antennal positioning in bees

In parallel to the above work, we had also initiated research on bees which allow us to conduct more difficult behavioral assays. At the National Center for Biological Sciences campus, we set up an apiary in which we trained the bees to find their way from the apiary, to a feeder placed at the upstream end of a custom wind tunnel situated in the laboratory. As they flew upwind towards the feeder in variable air flow, we tracked their antennal angles and flight trajectory with two high-speed video cameras and measure how they positioned their antennae during free flight when confronted by frontal airflow. These data showed that the animals actively positioned their antennae during flight and their antennal positions were a function of their speeds.

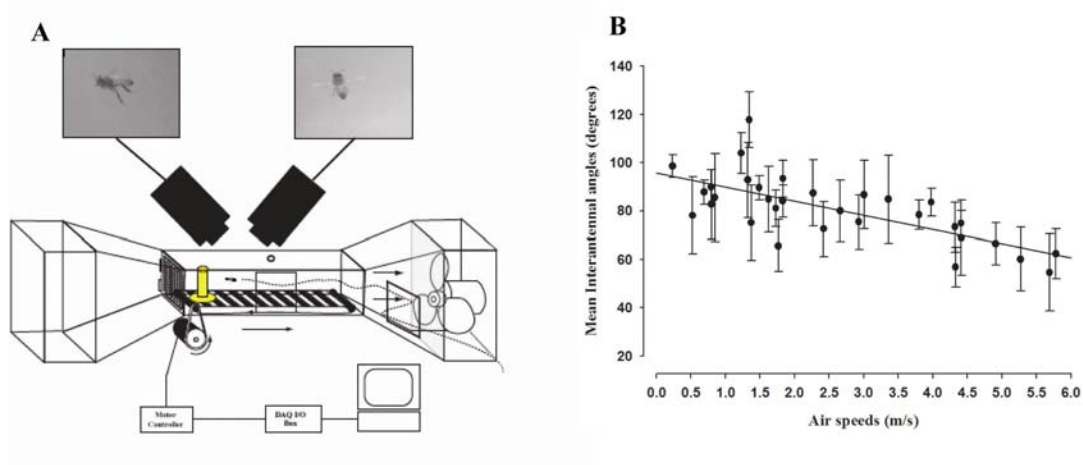


Figure 3: (A) Wind tunnel setup for free flight experiments with bees. (B) Graph showing the relationship between antennal angles and air speeds. Each point here is an individual bee. Airspeeds vs mean interantennal angles. The mean interantennal angles are negatively correlated with increasing airspeeds of the bee, with an r^2 value of 0.499, $p < 0.001$, $n = 32$. Randomization tests indicate that the correlation is significant.

Over the last year, we have extended this data set to now include data for antennae of individual insects are tracked through a flight bout under conditions of variable visual stimuli. This strategy enables us to behaviorally alter the speed of the honey bees using their natural behavioral responses to visual patterns. These results reiterate our conclusion that antennal positions are adjusted according to the air speeds (but not ground speeds), thus indicating that bees must have a way of independently assessing the flow of air over their body. What sensory structures mediate this air flow sensing and how do they connect to the antennomotor system? We are currently investigating this question in greater detail.

Antennal positioning in other insects: a broad survey of Bohm's bristles:

How general are our conclusions when applied to other insect taxa? We undertook a broad Scanning Electron Microscopy (SEM) survey of the antennae in diverse insects to look to presence or absence of Bohm's bristles as a first step towards addressing this question. As seen from Figure 4, Bohm's bristles are present in all major clades of Neopteran insects but not in the immediate outgroup. This suggests that they are an ancestral feature to all Neopteran insects and probably secondarily lost (or unidentifiably modified in a few cases such as Hemiptera and Diptera). We

plan to continue to populate this figure with further SEM imaging of the unsurveyed insects as well as confocal imaging of the patterns of neural arborization of the identified structures to determine if the underlying circuitry is also conserved.

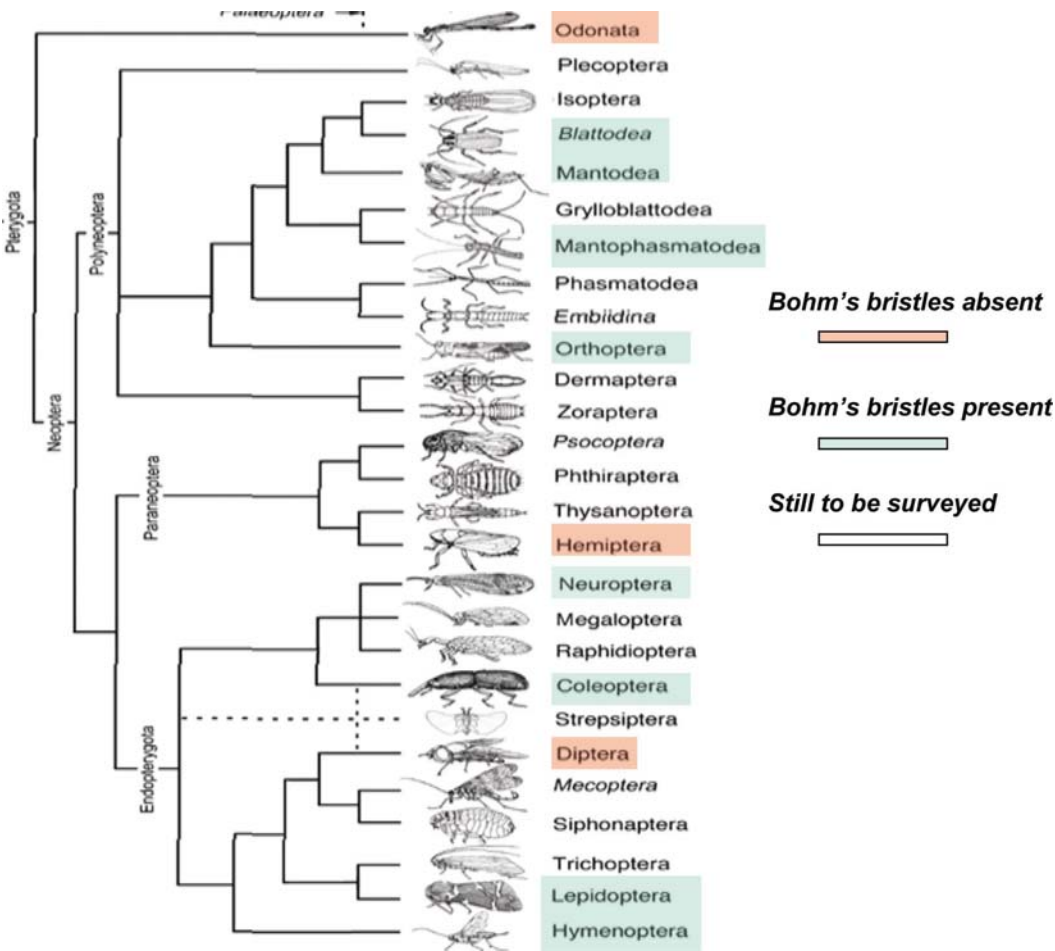


Figure 4: Presence and absence of Bohm's bristles mapped on to an insect phylogeny to indicate the generality of the mechanosensory structures for antennal positioning response.

Coordination of wings and halteres in the soldier fly, *Hermetia illucens*:

In most Dipteran insects, halteres move anti-phase to the wings during flight. To understand how the wings and the halteres maintain coordination during flight, we recently began a series of experiments to investigate the neurobiology underlying the coordination of wings and halteres.

During the course of these experiments, we discovered that the coordination of driven by mechanical coupling between the wings and halteres. Specifically, we

found that a minor structural element of the thorax called the scutellum mediates the wing-wing coordination, whereas another independent structural element connects the wings and the halteres. We have tested these hypotheses by severing these links and showing that it disrupts the respective synchrony in their motion. In both the wing-wing and wing-haltere case, the disruption causes a change in the phase relationship between these appendages but not the basic rhythm suggesting that they are independently actuated but their coordination requires mechanical connectivity.

These results help explain some very puzzling aspects of insect flight. For instance, certain Dipteran insects such as mosquitoes flap at 450 Hz and are yet able to precisely coordinate their wings and halteres. Because these time scales are too fast for the nervous system, it was not clear how these insects achieved such high frequencies. We now know that the nervous system is not involved in this coordination, nor is it limited by speeds of neural conductance. Rather, the limitation is physical, in this case the speed of sound in the cuticle, which is more than adequate to assure tight coordination between wings and halteres.

Location of odor sources in the fruit fly, *Drosophila melanogaster*

Our preliminary experiments had established that fruit flies use both visual and olfactory cues to pinpoint the location of an odor source. This is most evident in the moments before a fly lands on a food source when a fly undergoes a brief hovering phase that corresponds spatially to the location of odor source. When presented with a number of visual objects only one of which is also an odor object, the fly hovers over each object until, presumably, it encounters the visual object that presents the strongest odor concentration. To test this hypothesis, we have set up a wind tunnel in which we can conduct flight trials that challenge the fly to find an odor source when it is not in proximity of the visual source. Thus, we uncouple the visual and odor stimuli. These experiments lead to the following results:

1. When visual cues are absent, flies approach the odor objects directly but they make several errors in pinpointing the location of the odor.
2. When odor and visual cues are associated and coincident in space, flies approach the objects directly and make very few errors in locating the odor source.

3. When there is a small gap between the odor and visual cues, flies usually approach the visual object first and make a course correction to the land on the odor object (correct landing), or else they land on the visual object (mislanding).
4. As the gap between the odor and visual cues increases, flies make fewer mislandings, suggesting that their initial approach must be guided by odor but their search near the source is guided by vision.

Landing behavior in the housefly, *Musca domestica*

We were previously able to develop an assay in which we could get a housefly to repeatedly land on an object of our choice, which was within a small volume of interest. Thus, we filmed the landing behavior at fairly good resolution, as well as quantified the wing kinematics using custom MATLAB software developed by Dr. Ty Hedrick (UNC Chapel Hill). Using such methods, we can now address how wing kinematics causes body turns. This work will be done in collaboration with Dr. Xinyan Deng, with whom we have a long-standing collaboration. They have developed a dynamics model that calculates the forces generated by a flapping wing, using a quasi-steady aerodynamic model that I had developed as a graduate student. The forces and torques resulting from altered kinematics will allow us to address how the fly changes its wing motion to generate specific maneuvers.

This work will now continue with added assays for pitch and yaw, in addition and landing and we also hope to extend it to study insect-insect interactions, such as territorial chases and predator-prey interactions.

Invited Lectures & Symposia (2010-2011)

Title: The mechanisms of fast sensorimotor responses in flying insects

Plenary speaker, Insect Flight Symposium, CAnMove program, Lund University, Sweden (Aug 29, 2011)

Title: Mechanisms of fast sensorimotor responses in insect flight control.

Invited Speaker: Eglin Air Force Base, US Air Force, Florida, USA (19 April, 2011) and Wright-Patterson Air Force Base, Dayton, Ohio, USA (21 April, 2011)

Title: Antennal positioning in flying insects

Invited speaker, International Conference of Intelligent Unmanned Systems, Bali, Indonesia (Nov 4, 2010)

Title: Positioning the Sensors: Reflexes and Preflexes

Invited speaker, Tata Institute of Fundamental Research, Mumbai October 21, 2010

Title: How do insects fly?

Department of Physics, Mysore University, Mysore Sept 16 2010

Title: Physical and Neural basis of insect flight

Invited speaker, Umea University, Sweden, August 27 2010

Title 1: Understanding Behavior 1: A Comparative look at Nervous Systems

Title 2: Understanding Behavior: Flight as an example

Title 3: Behavioral and Biomechanical insights into Insect Flight

Instructor, ICTP Workshop on Development and Evolution of Nervous Systems

International Centre for Theoretical Physics, Trieste, Italy, August 13 2010

Title: Understanding Behavior: Flight as an example

Invited speaker, Maggot meeting, NCBS Bangalore (24 July 2010)

Title: Behavioral and Biomechanical insights into Insect Flight Behavior

Invited Speaker, Wright State University, Dayton, Ohio, USA (26May 2010)

Publications (2010-2011)

(* author for correspondence)

Sane, S.P.* and Singh, A.K. (2011) Water Movement in Vascular Plants: A Primer. **Journal of the Indian Institute of Science (in press)**

Zhao, L.; Deng, X.* and Sane, S.P.* (2011). Modulation of leading edge vorticity and aerodynamic forces in flexible flapping wings. **Bioinspiration and Biomimetics (in press).**

Singh, A.K, Prabhakar, S and Sane, S. P.* (2011). The biomechanics of fast prey capture by aquatic bladderworts. **Biology Letters, 7, 547-550.**

Sane SP* (2011) Steady or Unsteady? Uncovering the Aerodynamic Mechanisms of Insect Flight. **J. Exp. Biol., 214, 349-351**

Sane SP*, Srygley RB, Dudley R (2010) Antennal regulation of migratory flight in the neotropical moth *Urania fulgens*, **Biology Letters, 6: 406-409**

Zhao L, Huang Q, Deng X* and Sane, SP* (2010) Aerodynamic effects of flexibility in flapping wings. **The Journal of Royal Society Interface 7: 485-497**

Sane SP* and McHenry MJ (2009) The biomechanics of sensory organs, **Integrative and Comparative Biology, 49(6):i8-i23;**

Meeting Abstracts (2010-2011)

Krishnan, A., Sudarsan, S., Prabhakar, S., and Sane, SP*. Antennal positioning in flying insects. (9th International Congress of Neuroethology, Salamanca, Spain, August 2010).

Deora, T., and Sane, SP*. Mechanical coupling of the wings and halteres in the black soldier fly, *Hermetia illucens*. (9th International Congress of Neuroethology, Salamanca, Spain, August 2010).

Roy, T., and Sane, SP*. Mechanosensory-Visual integration during the honey bee flight. (9th International Congress of Neuroethology, Salamanca, Spain, August 2010).

Sane, SP*. The tale of two mechanosensors: antennal role in insect flight (Ann. Meet. of Soc. for Integrative and Comparative Biology (SICB). Boston, MA Jan., 2009)